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(54) Animal feed additive comprising enzyme and amino acid

(57) A concentrated animal feed additive containing a plant tissue-destruction enzyme and at least one essential amino acid, effective for increasing milk yield, improving milk qualities, promoting growth, improving the meat qualities and elevating the breeding efficiency of animals. The enzyme may comprise at least one of cellulase, xylanase, mannanase, laminarinase, pectinase, amylase, ligninase, protease and dextranase, and the amino acid selected from lysine, methionine, threonine and tryptophan.

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ANIMAL FEED ADDITIVE

FIELD OF THE INVENTION

This invention relates to an animal feed additive which is effective for increasing milk yield, improving milk qualities, promoting growth, improving meat qualities or elevating the breeding efficiency of animals, in particular, those having a ruminant stomach (hereinafter called ruminants).

BACKGROUND OF THE INVENTION

It has been known to add enzyme compositions containing enzymes having plant tissue-destruction activity such as cellulase to cattle feeds in order to increase the milk yield and to improve the milk qualities of milk cows [Biotechnology Letters, 9 (5), 369 (1987); and U.S.Patent 4,144,354].

It has previously been considered that it is sufficient to supply ruminants with a feed mainly comprising roughage without providing essential amino acids since microorganisms produce essential amino acids in the rumen of ruminants. With the spread of the feeding of milk cows capable of providing an increased milk yield in recent years, however, it seems that the essential amino acids produced by microorganisms *in vivo* cannot provide a sufficient amount of protein. It is, therefore, necessary to supply a definite amount of non-digestible proteins

which would pass through the rumen. For example, attempts have been made to add protected amino acids to feeds [J. Dairy Sci., 69, 2348 (1986)]. However, none of these attempts have been successfully put into practical use, since the effects thus achieved are unsatisfactory and, furthermore, the protected amino acids are expensive. It is also reported to orally administer a liquid composition containing amino acids to ruminants (Japanese Published Unexamined Patent Application No. 255047/90). However this method suffers from problems including decomposition of the amino acids contained in the liquid composition as well as difficulty in administration and feeding.

The need exists for an inexpensive animal feed additive of good quality which is effective for increasing milk yield, improving milk qualities, promoting growth, improving meat qualities or elevating the breeding efficiency of ruminants.

SUMMARY OF THE INVENTION

The present inventors have found that oral administration of a feed with an animal feed additive which contains an enzyme having plant tissue-destruction activity together with one or more essential amino acids, is highly effective for increasing milk yield, improving milk qualities, promoting growth, improving meat qualities or elevating the breeding efficiency of the animals.

An object of the present invention is to provide a concentrated animal feed additive which consists essentially of an enzyme having plant tissue-destruction activity and at least one essential amino acid.

DETAILED DESCRIPTION OF THE INVENTION

Any enzyme having plant tissue-destruction activity, such as fibrinolytic activity, CMCase activity, saccharific activity, proteolytic activity and the like, can be used in the present invention, so long as the effects of the present invention can be achieved thereby.

Examples of suitable enzymes include cellulase, hemicellulases such as xylanase and mannanase, laminarinase, pectinase, amylase, ligninase, protease and dextranase. These enzymes or mixtures of them may be used alone or in combinations of two or more. A preferred example of an enzyme-containing mixture having plant tissue-destruction activity is obtained by incubating a microorganism of Basidiomycetes belonging to the genus Fomitopsis, Irpex or Ascomycetes belonging to the genus Trichoderma.

A specific example thereof include Driselase® (manufactured by Kyowa Hakko Kogyo Co., Ltd.) which is an enzyme composition obtained by culturing Basidiomycetes belonging to the species Irpex lacteus having plant tissue-destruction activity, in particular, intense plant cell

wall-destruction activity such as activities of cellulase, laminarinase, xylanase, pectinase, amylase, protease and dextranase (U.S.Patent 4,144,354).

Examples of the essential amino acids to be used in the present invention include valine, leucine, isoleucine, threonine, phenylalanine, tryptophan, methionine, lysine, histidine, arginine and salts thereof. Lysine, methionine, threonine and tryptophan are particularly suitable.

Any essential amino acid obtainable by synthesis or fermentation may be used. Further, for example, protected amino acids coated with an organic polymer or protected amino acid derivatives may be employed. Preferred protected amino acids are those which can liberate essential amino acids in the rumen or can be converted into essential amino acids.

The animal feed additive of the present invention may be given to an animal at a dose of from 0.01 to 0.5 g of amino acid, per kg body weight of the animal per day, which corresponds to a fiber-destructive potency of from 0.8 to 80 in terms of enzymes, per kg body weight of the animal per day. The unit of an enzyme having plant tissue-destruction activity used herein means "fiber-destructive potency" determined in accordance with the "fiber-destructive potency test" [Extra Edition No. 28 of Official

Gazette, pages 8 - 9, March 20, 1990, published by Printing Bureau of the Japanese Ministry of Finance].

The fiber-destructive potency test can be carried out as follows.

A sample is accurately weighed and dissolved in 1 M acetic acid/sodium acetate buffer having a pH optimal for the enzyme contained in the sample. A 5 ml portion of the sample solution is charged into an L tube and allowed to stand for 5 minutes at 37 ± 5 °C. Immediately after two pieces of filter paper (1 cm x 1 cm) are added thereto, the tube is shaken at 65 rpm, at an amplitude of 60 mm and at 37 ± 0.5 °C. The period of time required for completely destructing filter paper into fine fibers is measured and the fiber-destructive potency unit is calculated in accordance with the following equation.

$$\text{Fiber-destructive potency unit per gram} = \frac{1}{T \times W} \times 1{,}000$$

T: average time (min) required for completely destructing filter paper (except for the longest and the shortest)

W: amount of the sample (g) in 5 ml of the sample solution

Examples of the dose of each component in the feed additives per animal per day are given below.

(1) Milk cow (average body weight: 500 - 600 kg)

enzyme	400 - 4,000 (fiber-destructive potency unit)
L-lysine hydrochloride	5 - 50 (g)
DL-methionine	1 - 10 (g]
L-threonine	0.2 - 5 (g)
L-tryptophan	0.1 - 3 (g)

(2) Cattle on rearing (calf) (average body weight: 100 - 200 kg)

enzyme	80 - 800 (fiber-destructive potency unit)
L-lysine hydrochloride	1 - 10 (g)
DL-methionine	0.2 - 5 (g)
L-threonine	0.05 - 3 (g)
L-tryptophan	0.02 - 2 (g)

(3) Fattening cattle (average body weight: 500 - 600 kg)

enzyme	400 - 4,000 (fiber-destructive potency unit)
L-lysine hydrochloride	5 - 50 (g)
DL-methionine	1 - 10 (g)
L-threonine	0.2 - 5 (g)
L-tryptophan	0.1 - 3 (g)

By animals we mean livestock and domestic animals such as cattle, goats, sheep, camels, deer and reindeer.

The feed additives according to the present invention may be formulated into, for example, a powder, pellets or an aqueous solution and given to the animals either alone or in the form of a mixture with other feeds.

According to the present invention, an animal feed additives, which are excellent in improving milk yield, improving milk qualities, promoting growth, improving meat qualities or elevating the breeding efficiency of animals when orally administered, can be obtained by adding an enzyme having plant tissue-destruction activity and one or more essential amino acids to a traditional animal feed.

To further illustrate the present invention in greater detail, the following examples are given, which are not to be construed to limit the scope of the present invention.

EXAMPLE 1

Improvement in milk yield and milk qualities of milk cows

30 female lactating adult Holstein milk cows were divided into six groups (A, B, C, D, E and F) of 5 cows each. In a preliminary test, all 30 cows were fed with a traditional feed of the composition shown in Table 1 for 10 days (the preliminary test period). Next, each of six different pre-mix feeds containing the additives according to the present invention listed in Table 2 was mixed with the traditional feed of Table 1 in such a manner as to give a dose of 100 g of pre-mix per animal per day. Then the cows were fed with the feed thus obtained for 30 days (the

main test period). The milk yield was measured and, milk fat percentage, non-fat solid percentage of the milk and milk protein percentage were determined with Milko-scan 133 (AS. N. Foss. Electric) and the results reported in Table 3.

TABLE 1

Composition of traditional feed	(kg/animal/day)
Corn	4.3
Barley with husk	3.6
Heated soybean	1.5
Cotton seed meal	1.5
Beet pulp	3.3
Soybean meal	1.5
Corn gluten feed	1.0
Lucerne	4.0
Hay cube	1.3
Sudan grass	2.6
Oat	1.3

TABLE 2

Composition of pre-mix feeds (% by weight)

	A	B	C	D	E	F
L-lysine hydrochloride	10	10	10	10	0	0
DL-methionine	2	2	2	2	0	0
L-threonine	0.5	0.5	0	0	0	0
L-tryptophan	0.5	0.5	0	0	0	0
Driselase*	1	0	1	0	1	0
Glutamic acid fermentation meal	34	34	34	34	34	34
Citric acid	2	2	2	2	2	2
Vitamin/mineral premix	10	10	10	10	10	10
Algae powder	10	10	10	10	10	10
Lucerne meal	20	20	20	20	20	20
Rice bran	10	11	11	12	23	24
Total	100	100	100	100	100	100

*: Enzyme activity in Driselase =
800 fiber-destructive potency unit/g.

TABLE 3

Test	Item							
	Milk yield (kg/day)		Milk fat percentage		Non-fat solid percentage		Milk protein percentage	
	P*1	M*2	P	M	P	M	P	M
Group A	27.0	29.9	3.4	3.8	8.3	9.4	3.0	3.5
Group B	27.3	28.6	3.4	3.5	8.3	8.6	3.0	3.2
Group C	27.5	30.0	3.4	3.7	8.3	9.2	3.0	3.3
Group D	27.3	28.5	3.4	3.5	8.3	8.5	3.0	3.1
Group E	27.2	28.0	3.4	3.5	8.3	8.5	3.0	3.1
Group F	27.0	27.1	3.4	3.4	8.3	8.3	3.0	3.0

*1: P means the preliminary test period.

*2: M means the main test period.

(Expressed in the average in each test period.)

Table 4 shows the percent gain in each item during the main test period based on the average in the preliminary test period (prior to incorporating the premix).

TABLE 4

	Milk yield percentage	Milk fat percentage	Non-fat solid percentage	Milk protein percentage
Group A	10.7	11.8	13.3	16.7
Group B	4.8	2.9	3.6	6.7
Group C	9.1	8.8	10.8	10.0
Group D	4.4	2.9	2.4	3.3
Group E	2.9	2.9	2.4	3.3
Group F	0.4	0.0	0.0	0.0

These results indicate that the administration of the feed additives of the present invention (groups A and C) to milk cows resulted in an increase in milk yield and improvement in milk qualities.

EXAMPLE 2

Growth promotion of calves

30 female Holstein calves aged 5 to 6 months were divided into six groups (A, B, C, D, E and F) of 5 calves each. In a preliminary test, all calves were fed with a traditional feed comprising 5 kg/day of a roughage (dry pasturage) and 4 kg/day of a concentrated feed for grower, which is referred to as the "traditional feed A" hereinafter, for 10 days (the preliminary test period). Next, each of six different pre-mix feeds containing the additives according to the present invention listed in the above Table 2 was mixed with the traditional feed A in such

a manner as to give a dose of 50 g of pre-mix per animal per day. Then the calves were fed with the feed thus obtained for 90 days (the main test period). The body weight gain during each test period was measured.

The body weight gain ratios based on the control group (group F) were determined according to the following equation.

$$\text{Body weight gain ratio (\%)} = \frac{\text{Body weight gain of each group}}{\text{Body weight gain of control group}} \times 100 - 100$$

Table 5 shows the results.

TABLE 5

	Body weight at the end of pre. test (kg/animal)	Body weight at the end of main test (kg/animal)	Body weight gain ratio (%)
Group A	165	290	21.4
Group B	165	277	8.7
Group C	164	279	11.7
Group D	170	280	6.8
Group E	163	271	4.9
Group F	170	273	0.0

These results indicate that the growth of the calves was promoted by feeding them the feed additives of the present invention (groups A and C).

EXAMPLE 3

Improvement in meat texture of fattening cattle

Three months before shipping, 15 Holstein oxen aged 32 months were divided into three groups (G, H and I) of 5 oxen each. In a preliminary test, all oxen fed with a traditional feed comprising 2 kg/day of a roughage (rice straw) and 10 kg/day of a concentrated feed for finisher, which is referred to as the "traditional feed B" hereinafter, for 10 days (the preliminary test period). Next, each of three different pre-mix feeds containing the additives according to the present invention listed in the following Table 6 was mixed with the traditional feed B in such a manner as to give a dose of 100 g of pre-mix per

animal per day. The oxen were fed with the feed thus obtained for 90 days (the main test period). The body weight during each test period was measured. Further the rib eye area (cm^2) at the slaughter/shipping were measured by dissecting light split carcass between the 6th rib and the 7th rib and measuring the area of musculus longissimus thoracis (rib eye) of the section.

The body weight gain ratios based on the control group (group I) were determined according to the following equation.

$$\text{Body weight gain ratio (\%)} = \frac{\text{Body weight gain of each group}}{\text{Body weight gain of control group}} \times 100 - 100$$

Table 7 shows the results.

TABLE 6

Composition of pre-mix feeds (% by weight)	G	H	I
L-lysine hydrochloride	10	0	0
DL-methionine	2	0	0
L-threonine	0.5	0	0
L-tryptophan	0.5	0	0
Driselase*	1	1	0
Glutamic acid fermentation meal	34	34	34
Citric acid	2	2	2
Vitamin/mineral premix	10	10	10
Algae powder	10	10	10
Lucerne meal	20	20	20
Rice bran	10	23	24
Total	100	100	100

*: Enzyme activity in Driselase =
800 fiber-destructive potency unit/g.

TABLE 7

	Body weight at the end of pre. test (kg/animal)	Body weight at the end of main test (kg/animal)	Body weight gain ratio (%)	Rib eye area (cm^2/animal)
Group G	616	736	18.8	45
Group H	612	721	7.9	42
Group I	620	721	0.0	40

These results indicate that the administration of the feed additives of the present invention (group G) increased the meat weight and enlarged the rib eye area of oxen before shipping.

EXAMPLE 4

Feed for milk cow

A feed for milk cow of the composition as listed in the following Table 8 was prepared and given to 5 female adult Holstein milk cows in an amount of 26,000 g/animal/day for 4 months. As a control, the same composition as in Table 8 except that L-lysine hydrochloride, DL-methionine, L-threonine and Driselase were replaced with the same amount of rice bran was prepared and given to cows of a control group.

At the initiation and completion of feed, the milk yield was measured and milk fat percentage, non-fat solid percentage and milk protein percentage were determined with Milko-scan 133 (AS. N. Foss Electric). The results are shown in Table 9.

TABLE 8

Composition of milk cow feed	(g)
Corn	4300
Barley with husk	3600
Heated soybean	1500
Cotton seed meal	1500
Beet pulp	3300
Soybean meal	1500
Corn gluten feed	1000
Lucerne	4000
Hay cube	1300
Sudan grass	2600
Oat	1300
L-lysine hydrochloride	10
DL-methionine	2
L-threonine	0.5
Driselase*	1
Glutamic acid fermentation meal	34.5
Citric acid	2
Vitamin/mineral premix	10
Algae powder	10
Lucerne meal	20
Rice bran	10
Total	26,000

*: Enzyme activity in Driselase =
800 fiber-destructive potency unit/g

TABLE 9

	Milk yield (kg/day)	Milk fat percentage	Non-fat solid percentage	Milk protein percentage
Invention initiation of feed	27.5	3.4	8.3	3.0
completion of feed	28.9	3.7	8.8	3.3
Control initiation of feed	27.3	3.4	8.5	3.0
completion of feed	27.4	3.4	8.5	3.0

The results in Table 9 indicates that the administration of the feed additives of the present invention (Table 8) increased milk yield and improvement in milk qualities.

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

CLAIMS

1. A concentrated animal feed additive which consists essentially of an enzyme having plant tissue-destruction activity and at least one essential amino acid.

2. The concentrated animal feed additive as claimed in Claim 1, wherein said enzyme having plant tissue-destruction activity is one or more substances selected from a group consisting of cellulase, xylanase, mannanase, laminarinase, pectinase, amylase, ligninase, protease, dextranase and mixtures of two or more thereof.

3. The concentrated animal feed additive as claimed in Claim 1, wherein said essential amino acid is selected from lysine, methionine, threonine and tryptophan.

4. The concentrated animal feed additive for ruminant animals as claimed in Claim 1.

5. A method for improving milk yield and milk quality, promoting growth, improving meat quality or improving the breeding efficiency of ruminants comprising orally administering to said ruminant an effective amount of a composition containing an enzyme having plant tissue-destruction activity in combination with at least one essential amino acid.

6. The method of Claim 5 in which the amount of amino acid or amino acids administered is from about 0.01 to about 0.5 g per kg of animal body weight per day and the

amount of enzyme administered is from about 0.8 to about 80 fiber destructive potency unit per kg animal body weight per day.

7. The method of Claim 5, wherein said enzyme having plant tissue-destruction activity is one or more substances selected from a group consisting of cellulase, xylanase, mannanase, laminarinase, pectinase, amylase, ligninase, protease, dextranase and mixtures of two or more thereof.

8. The method of Claim 5, wherein said essential amino acid is selected from lysine, methionine, threonine and tryptophan.

9. A method as claimed in claim 5, substantially as hereinbefore described in any one of Examples 1 to 4.

10. An animal feed additive as claimed in claim 1, substantially as hereinbefore described in any one of Examples 1 to 4.

11. The combined use of an enzyme having plant tissue-destruction activity and at least one essential amino acid for improving milk yield, improving milk quality, improving meat quality and/or improving the breeding efficiency of ruminants.

Patents Act 1977
Examiner's report to the Comptroller und r Section 17 (The Search Report)

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Rel vant Technical fields

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Databases (see over)
(i) UK Patent Office

(ii) ONLINE DATABASES: WPI

Date of Search
8 FEBRUARY 1993

Documents considered relevant following a search in respect of claims 1-11

Category (see over)	Identity of document and relevant passages		Relevant to claim(s)
X	CA 1071923 A	(CANADA PACKERS LIMITED) - especially Example (Table 1); Claims 1, 11	1-11
X	HU0T39576 A	(MUANYAGIPARI KI) - Derwent Abstract Accession No WPI 86-31831/48	1
X	JP 600156350 A	(MITSUI TOATSU CHEMICALS INC) - Derwent Abstract Accession No WPI 85-239819/39	1, 5
X	SU 0743668 A	(BLACK EARTH ANIMAL) - Derwent Abstract Accession No WPI 81-15126D/09	1-11
X	US 4533557 A	(NIPPON S DA CO LTD) - especially Claim 1	1, 5

Category	Identity of document and relevant passages	Relevant to claim(s)

Categories of documents

X: Document indicating lack of novelty or of inventive step.

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P: Document published on or after the declared priority date but before the filing date of the present application.

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